National Renewable Energy Laboratory
Innovation for Our Energy Future

NREL is a national laboratory of the U.S. Department of Energy
Office of Energy Efficiency and Renewable Energy
Operated by the Alliance for Sustainable Energy, LLC

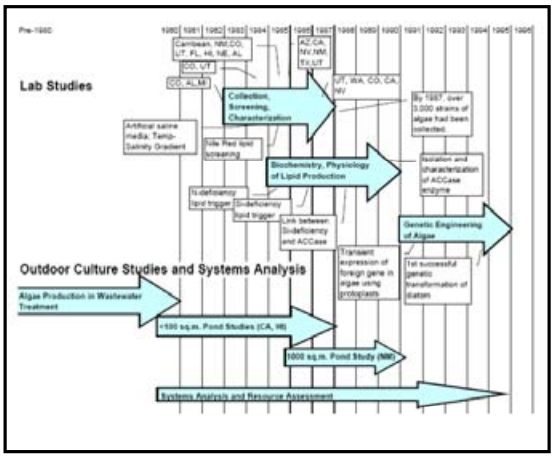
Current Status of the Department of Energy's Aquatic Species Program Lipid-Focused Algae Collection

Eric P. Knoshaug, Ryan Sestric, Eric Jarvis, Yat-Chen Chou, Philip T. Pienkos, and Al Darzins
National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO. 80401

Abstract

The Department of Energy's Aquatic Species Program (ASP) was funded from 1978 to 1996 in an effort to develop liquid transportation fuels from microalgae. During this time an extensive algae culture collection was amassed from water samples around the United States. Out of more than 3000 strains, 51 were well characterized in terms of growth and lipid production and these are described in the Microalgae Culture Collection (http://www.nrel.gov/docs/legosti/old/3079.pdf) and addendum (http://www.nrel.gov/docs/legosti/old/3079a.pdf). At the close of the ASP, a total of 297 strains of the original 3000, including 37 of the 51 strains listed in the Microalgae Culture Collection and addendum, were transferred to the Center for Marine Microbial Ecology and Diversity (CMMED) at the University of Hawaii (UH). The complete list of strains transferred is available in the ASP Closeout Report (http://www.nrel.gov/docs/legosti/fy98/24190.pdf). However, this list does not include some strains that were listed in the Microalgae Culture Collection yet are present in the UH collection. With the resurgence in interest in using microalgae as a feedstock for producing liquid transportation fuels, many inquiries have come up regarding the current status of this important strain collection. Currently, 23 of the 51 strains listed in the Microalgae Culture Collection and addendum are still extant and have been re-established at the National Renewable Energy Laboratory. We present here the current status of these strains including a microscopic characterization of potential lipid vesicles using neutral lipid specific dyes.

Overview of the DOE Aquatic Species Program Work



Aquatic Species Program collection sites within the continental United States.

Strains Listed in either the Microalgae Culture Collection or Addendum

AMPHO1
AMPHO27
AMPHO28
ANKIS1
BOEKE1
BOTRY1
CHAET1
CHAET6
CHAET9
CHAET10
CHAET14
CHAET15
CHAET38
CHAET39
CHAET58
CHAET59
CHAET61
CHAET63
CHLOR1
CHLOR2
CHLOR3
CYCLO1
CYCLO2
CYCLO4
ELLIP1
ENTOM3
FRANC1
GREEN3
ISOCH1
MONOR1
MONOR2
NANNO2
NANNO12
NANNP1
NANNP2
NAVIC1
NAVIC2
NAVIC6
NAVIC7
NAVIC8
NAVIC24
NITZS1
NITZS2
NITZS13
NITZS14
NITZS26
OOCYS1
PHAEO1
PHAEO2
PLUER1
TETRA1
TETRA4
THALA2
THALA6

Currently Extant Strains

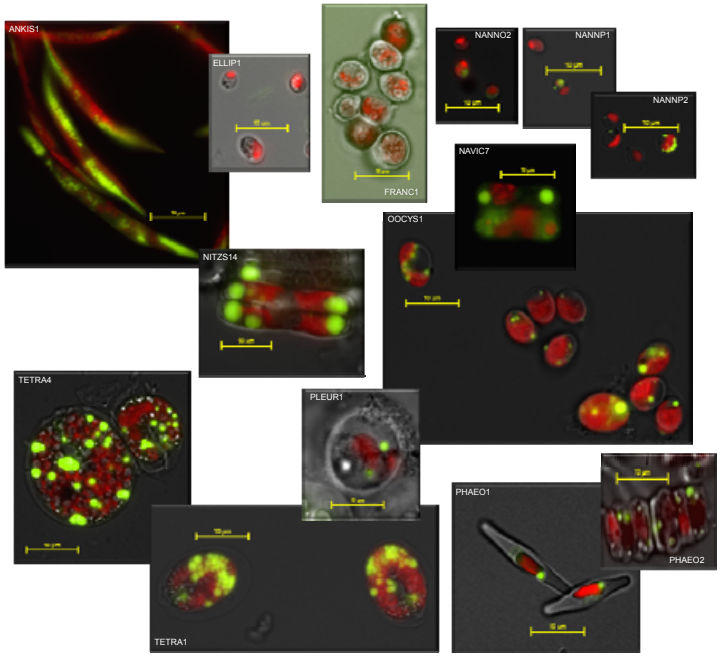
ANKIS1
CHAET6
CHAET59
CHLOR1
CHLOR2
CYCLO1
ELLIP1
FRANC1
MONOR1
MONOR2
NANNO2
NANNP1
NANNP2
NAVIC2
NAVIC7
NAVIC24
NITZS1
NITZS13
NITZS14
OOCYS1
PHAEO1
PHAEO2
PLUER1
TETRA1
TETRA4

This list shows the strains that were carefully investigated during the Aquatic Species Program and those that were transferred to the University of Hawaii at the close of that program. The green box shows the strains that still exist and are currently available from the University of Hawaii Marine Microbial Culture Center.

Not on list: of transferred strains in the close-out report. Lost prior to transfer to the University of Hawaii.

Endogenous Lipid Vesicles

Algal cells were stained with the neutral lipid specific dye, BODIPY 493/503. The stained cells were excited with 480 nm light and the pictures show the juxtaposition of the chlorophyll containing chloroplasts (red) and the putative lipid vesicles (green). When excited with 480 nm light, chloroplasts emit at ≥630 nm while BODIPY stained vesicles emit at 515 nm. These micrographs were taken of cells in the early stationary phase and have not been specifically stressed for lipid production.



Long Term Storage via Cryo-Preservation

Algal strains that have been re-established at NREL are in the process of being cyro-preserved in an effort to reduce the work-load associated with maintaining an algae collection and to prevent un-intended loss or genetic drift, a risk with frequent transfer. The cryo-freezer uses liquid nitrogen and cultures are stored at -195ºC in the vapor phase. We are now beginning to freeze and evaluate strains for survival.

Conclusion

An impressive collection of microalgae was assembled under the Department of Energy's Aquatic Species Program. Maintenance of microalgal cultures over the long term is challenging requiring frequent transfers exposing the cultures to the risks of contamination and genetic drift. The loss of many strains from the collection over time highlights the difficulties involved in maintaining algal culture collections over the long term. New methods and technologies are continually being tried and cyro-preservation, according to the literature, seems a good candidate for some species for long term storage. Although many of the strains have been lost over time, promising candidates for the production of lipids still exist from the original ASP culture collection.

Acknowledgments

The authors acknowledge the University of Hawaii for maintaining the NREL ASP algae collection.

NREL/PO-510-45788
May 3-6, 2009 • San Francisco, CA